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Systemic Effects of Inhaled Ultrafine Particles in Two Compromised, Aged Rat Strains

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Epidemiological studies associate morbidity and mortality with exposure to particulate air pollution in elderly individuals with existing cardiopulmonary disease. These associations led to the hypothesis that inhaled particles can exert adverse effects outside of the lung, particularly on the cardiovascular system. We tested this hypothesis by examining the pulmonary and peripheral effects of inhaled ultrafine carbon particles in old rats that were injected with endotoxin (lipopolysaccharide, LPS) to model systemic gram-negative bacterial infection. Fischer 344 rats (23 mo) and spontaneously hypertensive (SH) rats (11–14 mo) were injected with LPS (2 mg/kg, ip) immediately before being exposed to inhaled ultrafine carbon particles for 6 h (150 $\mu g/m^3$, CMD = 36 nm). Controls were injected with sterile saline or were sham exposed. Twenty-four hours after LPS injection, bronchoalveolar lavage (BAL) fluid, cells, and blood were obtained to assess endpoints of inflammation, oxidant stress, coagulability, and the acute-phase response. LPS did not cause an influx of neutrophils (PMNs) into the alveolar space, but did increase the number and percentage of circulating PMNs and the concentration of plasma fibrinogen in both rat strains. Inhaled ultrafine particles did not induce lung inflammation in either rat strain. In both strains, ultrafine particles (UFP) were found to decrease the number of blood PMNs, increase the intracellular oxidation of a fluorescent dye (DCFD) in blood PMNs, and affect plasma thrombin–anti-thrombin (TAT) complex and fibrinogen levels. UFP were also found to interact with ip LPS with respect to plasma TAT complex levels and blood PMN DCFD oxidation. Differences between the two rat strains were also found for TAT complex levels, BAL cell reactive oxygen species release, and DCFD oxidation in both BAL macrophages and blood PMNs. These results suggest that inhaled ultrafine carbon particles inhaled at concentrations mimicking high episodic increases in urban air can exert extrapulmonary effects in old rats and that they can change the systemic response to an inflammatory stimulus.

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Epidemiological studies consistently demonstrate an association between morbidity and mortality in susceptible populations and low ambient particle concentrations. Among the most susceptible are individuals >65 yr old with cardiopulmonary disease (Schwartz & Dockery, 1992; Burnett et al., 1995; Schwartz & Morris, 1995). However, the lack of both specificity and plausible mechanisms have challenged the validity of these associations. Although epidemiological studies focus mainly on the mass concentration of fine- and coarse-mode particles, the size distribution of ambient air particles is

trimodal and includes ultrafines, which have the highest number concentration by a factor of ~60,000 compared with the coarse-mode particles (Pekkanen et al., 1997). Ultrafine particles (UFP) also have high predicted fractional deposition in the alveolar regions of the lung (International Committee on Radiological Protection (ICRP), 1994). Furthermore, data from our laboratory suggests that ultrafine as compared to fine-mode particles (TiO_2) have a greater potency per unit mass for inducing lung inflammation (Oberdörster et al., 1994; Osier et al., 1997).

A current hypothesis about inhaled particles is that they can have systemic effects, either directly or indirectly, thus exacerbating underlying disease. Peters and colleagues (1997) showed increases in plasma viscosity in association with high air pollution episodes; furthermore, increases in heart rate in response to particles were found for those individuals with the highest viscosity. In addition, studies with concentrated ambient particles (CAPs) have reported increases in plasma fibrinogen in humans (Ghio et al., 2000) and in the numbers of circulating neutrophils (PMNs) in canines (Clarke et al., 2000). These data suggest that exposure to pollution-associated particles can alter circulating inflammatory cells and coagulability. If such changes can be caused by airborne particulates, then it is reasonable to assume that one would be at greater risk if such markers were already elevated, as may be the case in susceptible subgroups.

Lipopolysaccharide (LPS, endotoxin) is a component of gram-negative bacterial cell walls and is both ubiquitously present in the environment and released in vivo during infection. When administered systemically, it induces the expression of numerous molecules involved in inflammation, oxidative stress, the acute-phase response, and coagulation (Albelda, 1991; Sonesson et al., 1994; Hara et al., 1997; Jourdain et al., 1997; Wong & Wispe, 1997; Shinguh et al., 1998). Given these effects, we considered it to be a good candidate for inducing a compromised cardiopulmonary system and thus developed a model using intraperitoneal LPS to prime respiratory tract cells from the circulatory side of the lung and to induce an acute-phase response, such as would occur during systemic infection. The intent was to model those individuals who already have a compromised cardiopulmonary system prior to exposure to episodic elevations in ambient air particulate material.

The epidemiological data guided the selection of animal models for the studies described herein. The elderly are at greatest risk for adverse effects of particulate matter, perhaps due to cellular and biochemical changes that occur during aging. These changes may tip the balance between oxidant and antioxidant processes toward a state of oxidative stress. Indeed, data from our own laboratory showing increased lavage neutrophils and respiratory burst activity in old versus young animals supports this notion (Elder et al., 2000). Aside from aged rats, the other susceptibility model we chose for these studies was the spontaneously hypertensive (SH) rat. This is a genetic model of systemic hypertension with cardiomyopathy that has many similarities to the human disease; since hypertension develops very early, these rats have many cardiovascular complications that are relevant to human cardiopulmonary disease (Kodavanti et al., 1998).

Given the likelihood of exposure to ambient UFP and their distinct biology, we hypothesized that they could be causally related to the adverse effects described by epidemiologists. We also proposed that systemic LPS will create a compromised cardiovascular system, which could exacerbate the lung and systemic responses to inhaled particles. We therefore exposed rats to combinations of inhaled laboratory-generated ultrafine carbonaceous particles and intraperitoneal LPS. We chose two different animal models for our studies: F-344 rats that were already in a state of greater fragility due to advanced age (23 mo), and aged SH rats (11–14 mo), which are more fragile due to both their age and cardiovascular disease. The endpoints that we measured in these studies are directly related to lung and blood inflammatory cell oxidant stress, the acute-phase response, lung inflammation, and coagulability. The results from these studies shed light both on the extrapulmonary effects of inhaled UFP and on how they might exacerbate an already compromised cardiopulmonary system.

MATERIALS AND METHODS

Animals

Male Fischer 344 rats (Harlan; Indianapolis, IN) were aged in-house to 23 mo. Male SH rats (Taconic, Germantown, NY) were aged in-house to 11–14 mo. All animals were specific pathogen free, including from *Helicobacter*. Animals were housed individually in wire-bottom cages and given free access to food and water in a room with temperature and humidity control and a 12-h light–dark cycle. In total, 20 rats per strain were randomly distributed among 4 treatment groups with 5 rats each: (1) intraperitoneally (ip)-injected saline, inhaled air; (2) ip-injected saline, inhaled particles; (3) ip-injected LPS, inhaled air; and (4) ip-injected LPS, inhaled particles.

Exposures to Endotoxin and Ultrafine Carbon Particles

LPS was purchased from Sigma Chemical Co. (St. Louis, MO; L-9143, stock solution at 5×10^6 EU/ml) and diluted in 0.9% sterile, pyrogen-free saline prior to ip injection. Rats were injected with either LPS (2 mg/kg body weight) or sterile saline immediately prior to the beginning of the exposures to particles in compartmentalized, horizontal-flow whole-body chambers. Ultrafine carbon particles (150 $\mu g/m^3$, 6 h; count median diameter = 36 nm; particle classifier; TSI, Inc., St. Paul, MN) were generated in an argon-filled chamber using electric arc discharge (Palas Co., Germany) between two opposing ultrapure graphite rods. Electrostatic charge was brought to Boltzman equilibrium by a ^{210}Po source. Particle number and mass concentrations were measured at regular intervals (condensation particle counter, TSI; TEOM, Rupprecht and Patashnik, Albany, NY). Diluting air was added with a small amount of O_2 to maintain normal PO_2. Controls were sham exposed.

The dose of ip LPS was chosen from preliminary time-course and dose-response experiments that showed a doubling of the plasma fibrinogen and increased plasma interleukin (IL)-6 concentrations in response to 2 mg/kg LPS in aged F-344 rats (data not shown). The concentration for the UFP exposures was chosen to mimic episodic increases in ambient urban air, such as the ones reported by Brand et al. (1992). One can estimate that the predicted alveolar deposited dose in rats would be similar to an inhaled concentration of $\sim$74 μg/m^3 in humans (Yu & Xu, 1987; ICRP, 1994).

Bronchoalveolar Lavage and Cellular and Biochemical Parameters in Lavage Fluid

Rats were euthanized 24 h after exposure to ip LPS with an overdose of sodium pentobarbital (50 mg/100 g body weight, ip). Citrated blood was collected and the heart and lungs were removed en bloc. A small amount of blood was also collected from the jugular vein to determine the number and percentage of circulating peripheral blood neutrophils. Lungs were extensively lavaged with sterile saline (10 $\times$ 5 ml), keeping the first two lavages separate from the remaining ones. Bronchoalveolar lavage (BAL) cell viability (trypan blue exclusion), number, and the percentage of different cell types (Diff-Quik; Baxter Scientific, Edison, NY) were determined. Total protein concentration was measured as an indication of cytotoxicity and membrane permeability changes with reagents purchased from Sigma. The activities of the lysosomal enzymes, lactate dehydrogenase and β-glucuronidase, were measured in the BAL supernate using commercially available kits (Pierce Chemical Co., Rockford, IL; Sigma).

Measurement of BAL Cell-Derived Reactive Oxygen Species Activity

Respiratory burst activity was measured in lavage cell suspensions under both resting and phorbol myristate acetate (PMA, 1.5 $\times$ 10^{-7} M; Sigma)-stimulated conditions as an indication of released reactive oxygen species (ROS). BAL cells (10^5) were mixed with luminol (10^{-4} M; Sigma) in Krebs–Ringer HEPES buffer. Chemiluminescence was measured using a TD-20e luminometer (Turner Designs; Sunnyvale, CA) every 4 min for 20 min. Respiratory burst was measured within 3 h after cell isolation. A time-response curve for each sample both with and without PMA was constructed, and the area under these curves (AUC) was calculated using SigmaPlot (Jandel Scientific; San Rafael, CA) to obtain total respiratory burst activity.

Intracellular Reactive Oxygen Species Activity

Intracellular oxidation of 2′,7′-dichlorodihydrofluorescein diacetate (DCFD, 20 μM; Molecular Probes, Inc., Eugene, OR) was measured in alveolar macrophages (AMs) and peripheral blood PMNs. Cells were incubated with DCFD and PMA (50 μM; Sigma) in Krebs-Ringer phosphate buffer with Ca^{2+}, Mg^{2+}, and glucose (pH 7.3); all oxidative reactions were stopped by fixing the cells in 1% paraformaldehyde. BAL and blood cells were stained with phycoerythrin-labeled anti-CD45 (pan-leukocyte marker; Pharmingen B-D, San Jose, CA) to aid in cell separation. Red blood cells were lysed with buffer purchased from Becton-Dickinson (San Jose, CA). Samples were analyzed for forward- and side-scattering properties as well as green and red fluorescence intensity using a FACScan flow cytometer (Becton-Dickinson). The green (DCFD) fluorescence intensity of each sample was determined from a curve constructed with standardized beads (Flow Cytometry Standards Corp., San Juan, Puerto Rico) and expressed as mean equivalent standardized fluorescence (MESF) units.

Acute-Phase and Coagulability Parameters in Peripheral Blood

Spun hematocrit was measured to determine if exposure had any effects on packed red blood cell volume. Viscosity (whole blood and plasma) was measured as a function of sheer stress using a Brookfield cone and plate viscometer (Stoughton, MA) and expressed as minimum apparent viscosity (MAV), which has no units. In addition, the concentrations of fibrinogen, thrombin–anti-thrombin (TAT) complexes, and IL-6 in plasma were assessed. Fibrinogen was measured according to a heat precipitation method (Desvignes & Bonnet, 1981; Tan et al., 1995) that was adapted to 96-well microtiter plates in comparison to rat fibrinogen standards (Sigma). TAT complexes (Dade-Behring, Newark, DE) and IL-6; (BioSource International, Inc., Camarillo, CA) concentrations in plasma were measured via enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions. The TAT ELISA was made for human samples, but has cross-reactivity for rat complexes (Ravanat et al., 1995); the IL-6 ELISA was rat specific.

Data Analysis

The results were analyzed for statistically significant effects of each factor alone (main effects) and in combination (interaction effects) by two-way analysis of variance (ANOVA) using SigmaStat. The factors were the presence or absence of LPS and ultrafine carbon particles. Data were appropriately transformed if an analysis of residuals suggested deviations from the assumptions of normality and equal variance. Such analyses were also used to identify outliers, which were replaced with group means. Differences between groups were further analyzed using Tukey multiple comparisons. All comparisons were considered statistically significant when $p < .05$.

RESULTS

Neither inhaled ultrafine carbon particles nor ip LPS caused a significant increase in BAL fluid total cells or the percentage

of neutrophils in either rat strain. Similarly, there were no significant exposure-related alterations in total protein concentration or the activities of LDH and β-glucuronidase. The total cell number was elevated in both strains as compared to literature values for healthy, young rats; however, there were no treatment-related changes. The total protein concentration was somewhat higher in SHRs as compared to F-344 rats, probably due to increased leakiness of the lung epithelium and the presence of hemolyzed and nonhemolyzed blood (13 out of 20 "bloody" lavage fluid samples compared to 1 out of 20 from F-344 rats). These data are summarized in Table 1. Lung to body weight ratios were significantly increased (27%) in LPS-exposed F-344 rats, due, in part, to a 13% difference in body weight (not related to exposure, which resulted in only ~2% loss in body weight, or differences in age); no changes at all in this parameter were found for SHRs (data not shown).

Table 2 summarizes the measurements made in peripheral blood to gain information about treatment-related changes in inflammatory cell distribution (number and percentage of blood PMNs), coagulability (viscosity, TAT complexes), acute-phase responses (fibrinogen, IL-6), and the volume of packed RBCs. In both rat strains, ip LPS induced significant increases in both the number and percentage of circulating PMNs. However, when inhaled UFP were combined with ip LPS, the PMNs decreased, although this was only statistically significant for the blood PMN number in F-344 rats. In F-344 rats, the LPS/UFP interaction was such that the combined response was not different from controls. Plasma fibrinogen concentration was also increased by ip LPS treatment in both rat strains, although the magnitude of change was greater in SHRs. Interestingly, UFP by themselves (i.e. significant main effect) decreased plasma fibrinogen in SHRs. When inhaled UFP were combined with LPS, the response was somewhat blunted, but still significantly higher than control (saline/shams) values. In F-344 rats, there was also a decrease in fibrinogen that was driven by the interaction between LPS and UFP; in SHRs, the decrease was driven by the main UFP effect. Hematocrit was not altered by either treatment or their combination in either rat strain. Whole-blood viscosity was decreased in F-344 rats but increased in SHR by ip LPS treatment. UFP did not affect blood viscosity in SHR; although there was a small increase in saline-exposed F-344 rats, this did not reach statistical significance.

The results from analyses of TAT complexes revealed a striking strain difference in the level of response: With all of the exposure groups averaged, plasma TAT complexes in SHRs were 6.5 times higher than in F-344 rats. The direction of response following LPS treatment was also different in the two strains. LPS caused an overall increase in the concentration of plasma TAT complexes in F-344 rats that was further augmented by inhaled UFP (i.e., there was a significant interaction between LPS and UFP); UFP by themselves decreased the response. In SHRs, UFP alone significantly increased response and LPS decreased response; the blunting of response by LPS was such that the combined response was at control levels. The concentration of plasma IL-6 from F-344 rats in preliminary studies (data not shown) was one of the endpoints used to determine the dose of ip LPS used in these studies. As can be seen in the table, IL-6 was significantly increased by ip LPS in these rats; UFP did not change IL-6 levels by themselves or in combination with LPS. In SHRs, only the samples from filtered air-exposed rats had detectable levels of plasma IL-6, although highly variable; thus, statistical effects tests and group comparisons could not reliably be done.

The measurements of BAL and blood cell PMA-stimulated oxidant activities are summarized in Table 3. Although the levels of BAL cell ROS release were ~4-fold higher in SHRs as compared to F-344, the only exposure-related change found in chemiluminescence was a main effect for ip LPS in F-344 rats (reduced response). The oxidation of DCFD by intracellular ROS was measured in individual BAL AMs and blood PMNs via flow cytometry. As was true for chemiluminescence, the overall activity of intracellular ROS in BAL cells from aged SHRs was higher than that in old F-344 rats. In F-344, both UFP and systemic LPS had independent and significant effects on DCFD oxidation. These effects were in the opposite direction, however: particles decreased and LPS increased ROS activity. The significant main effect of UFP, for example, can be explained by the fact that the average of the two groups with particles is lower than the average of the two groups receiving filtered air, despite an apparent increase in saline/UFP-exposed versus saline/sham-exposed rats. There was also a statistically significant suppressive interaction between inhaled UFP and ip LPS such that the response was reduced to control levels. In SHRs, neither UFP nor LPS altered the activity of AM intracellular ROS, possibly because of the heightened basal levels present in these cells. Blood PMN intracellular ROS activity was lower in SH than in F-344 rats (by ~12-fold overall, ~19-fold in the controls alone), the opposite of what was found for BAL AMs. UFP in F-344 rats significantly increased blood PMN DCFD oxidation, but ip LPS caused a decrease; the combination of the two resulted in levels that were not different from control values. In contrast, ip LPS increased SHR blood PMN DCFD oxidation; furthermore, there was a significant interaction between LPS and UFP such that the combination of the two resulted in significantly higher levels as compared to either treatment alone.

Tables 4 and 5 summarize the statistically significant findings for BAL and blood parameters in old F-344 and SHRs, respectively. The arrows included in the tables under the p values, when present, indicate the direction of a main effect or interaction. The outcomes of the interactions are varied and result, for example, in no overall change (blood PMN number, Table 4), a suppression of response that still results in increases relative to control (fibrinogen, Table 4), or an enhancement of response above that attributable to either exposure component alone (TAT complexes, Table 4; blood PMN DCFD oxidation, Table 5).

TABLE 1
Summary of cellular and biochemical parameters in bronchoalveolar lavage fluid from F-344 rats and spontaneously hypertensive rats exposed to ip LPS and inhaled ultrafine carbon particles

	Total cells ($\times 10^7$)	% AMs	% PMNs	% Lymphocytes	% Viable	Protein (mg/ml)	LDH (nmol/min/ml)	β-Glucuronidase (nmol/min/ml)
F-344 rats								
Saline/sham	2.399 ± 1.997	95.11 ± 4.80	1.61 ± 0.91	3.31 ± 5.19	89.15 ± 2.68	0.349 ± 0.245	96.15 ± 56.05	0.396 ± 0.193
Saline/UFP	3.899 ± 3.015	96.59 ± 1.36	1.56 ± 1.19	1.90 ± 0.84	92.99 ± 2.02	0.314 ± 0.151	113.78 ± 107.61	0.251 ± 0.044
LPS/sham	1.366 ± 0.354	98.24 ± 1.61	1.38 ± 1.57	0.38 ± 0.23	89.55 ± 3.93	0.202 ± 0.034	81.67 ± 30.97	0.202 ± 0.047
LPS/UFP	1.921 ± 0.610	94.96 ± 7.46	0.84 ± 0.63	1.29 ± 0.94	90.35 ± 4.64	0.284 ± 0.075	127.54 ± 104.57	0.522 ± 0.354
SH rats								
Saline/sham	1.288 ± 0.129	95.15 ± 3.70	3.33 ± 2.54	1.49 ± 1.34	95.52 ± 1.87	0.632 ± 0.320	123.64 ± 70.94	0.431 ± 0.250
Saline/UFP	1.414 ± 0.223	95.86 ± 1.12	2.40 ± 1.57	1.73 ± 0.46	91.01 ± 3.05	0.468 ± 0.188	113.44 ± 46.59	0.317 ± 0.093
LPS/sham	1.547 ± 0.101	97.94 ± 1.58	1.51 ± 1.38	0.55 ± 0.26	94.27 ± 1.55	0.636 ± 0.249	120.03 ± 45.23	0.381 ± 0.133
LPS/UFP	1.466 ± 0.287	97.23 ± 0.40	1.68 ± 0.39	1.08 ± 0.43	93.21 ± 4.05	0.449 ± 0.236	113.66 ± 48.70	0.306 ± 0.128

Note. Values are means ± SD.

TABLE 2
Summary of parameters in peripheral blood from F-344 rats and spontaneously hypertensive rats exposed to ip LPS and inhaled ultrafine carbon particles

	Saline/sham	Saline/UFP	LPS/sham	LPS/UFP
F-344 rats				
%Blood PMNs	41.59 ± 3.77	37.95 ± 8.89	85.85 ± 4.56[c]	75.19 ± 9.31[c]
(number × 10^3/mm^3)	(1.73 ± 0.28)	(1.66 ± 0.49)	(5.94 ± 2.21)[c]	(2.71 ± 1.24)[a]
Fibrinogen (mg/ml)	5.10 ± 0.54	6.58 ± 0.62[c]	9.49 ± 0.62[c]	7.95 ± 1.82[a,c]
Hematocrit, %	39.8 ± 2.86	40.6 ± 4.12	39.0 ± 0.79	40.9 ± 4.07
Whole-blood viscosity (MAV)	3.34 ± 0.34	3.95 ± 0.61	2.91 ± 0.13[b]	3.04 ± 0.34[b]
TAT complexes (μg/L)	5.08 ± 0.69	1.67 ± 0.88[c]	5.45 ± 2.75	9.66 ± 3.02[a,c]
IL-6 (pg/ml)	57.0 ± 69.5	21.6 ± 30.3	268.9 ± 245.2[b]	183.7 ± 216.2[b]
SH rats				
%Blood PMNs	46.11 ± 6.01	41.86 ± 12.88	76.22 ± 11.31[c]	67.06 ± 18.23[c]
(number ×10^3/mm^3)	(2.96 ± 1.92)	(2.11 ± 0.96)	(8.61 ± 2.96)[c]	(7.52 ± 4.14)[a]
Fibrinogen (mg/ml)	5.88 ± 1.16	3.72 ± 1.24[c]	12.77 ± 1.67[c]	9.31 ± 1.60[a,c]
Hematocrit, %	37.9 ± 3.1	37.0 ± 2.6	39.0 ± 3.9	36.0 ± 6.2
Whole-blood viscosity (MAV)	3.32 ± 0.25	3.23 ± 0.12	4.22 ± 0.70[c]	4.07 ± 0.62[c]
TAT complexes (μg/L)	23.51 ± 9.63	71.30 ± 34.39[c]	19.08 ± 6.77	28.15 ± 6.76
IL-6 (pg/ml)	49.5 ± 103.5	ND	45.5 ± 83.8	ND

Note. Data are presented as means ± SD; ND indicates samples below the limit of detection for a given assay.
[a]Significantly different from LPS/shams, $p < .05$.
[b]Significant main effect of LPS, $p < .05$.
[c]Significantly different from saline/shams, $p < .05$.

DISCUSSION AND CONCLUSIONS

One of the goals of this work was to create a state of heightened cardiovascular sensitivity in an animal model. Preliminary studies (not shown) on the effects of ip LPS in F-344 rats showed that plasma fibrinogen and IL-6 concentrations were increased, suggesting activation of the acute-phase response and, possibly, coagulation. Both of these were increased here by ip LPS in F-344 rats, although whole blood viscosity itself was not altered. In SH rats, which are already in a more fragile state due to hypertension, plasma IL-6 levels did not change after ip LPS; however, both plasma fibrinogen and whole-blood viscosity increased after ip LPS exposure. These data suggest distinct acute-phase response pathways in the two animal models. LPS also increased the percentage and number of circulating PMNs in both rat strains. The oxidation of DCFD in blood PMNs was also affected by ip LPS in the two rat strains, but responses were in opposite directions, possibly due to a suppressed basal level of response in aged SHRs as compared to old F-344 rats.

In our previous work (Elder et al., 2000, 2002, 2003), we have demonstrated significant main effects of inhaled laboratory-generated UFP on inflammation in the lung, including increases in the percentage of BAL PMNs and in lavage cell ROS release; neither of these parameters were affected in this current study by UFP. One of the main reasons for the differences between this study and previous ones may be the number of experimental groups. The previous work utilized a multifactorial approach whereby both young and old animals were exposed to combinations of UFP and ozone following priming of respiratory tract cells with either inhaled LPS or instilled influenza virus. The total number of groups in these studies was 16, with a total n of 80; statistical comparisons were thus much more powerful.

Knowledge of how rats respond in comparison to humans with respect to inhaled particle effects will generate better experimental designs for future studies using animal models. Thus, another goal of these studies was to develop a set of parameters in rats that match those that are being measured in epidemiological and human clinical studies on the effects of particulate air pollution. Such studies focus on acute-phase responses, coagulability, changes in peripheral blood cellular distributions, and adhesion molecule expression with respect to extrapulmonary changes (Frampton et al., 2003). Seaton and colleagues (1999) found that packed RBC volume, plasma fibrinogen, and platelet counts decreased in elderly subjects with increasing mass concentrations of outdoor PM_{10}. In contrast, studies by Peters et al. (1997) detailed increases in plasma viscosity (which is driven by fibrinogen) in humans in association with increased total suspended particles. In a clinical study, Ghio et al. (2000) demonstrated that plasma fibrinogen also increased in response to concentrated ambient fine particles

TABLE 3
Oxidant release and intracellular activity in BAL and blood cells from old F-344 and spontaneously hypertensive (SH) rats

	Saline/sham	Saline/UFP	LPS/sham	LPS/UFP
Old F-344 rats				
PMA-stimulated chemiluminescence[c]	10.4 ± 5.5	7.2 ± 2.9	5.7 ± 4.1[b]	3.7 ± 1.0[b]
DCFD oxidation by BAL AMs (MESF, $\times 10^5$)	4.49 ± 0.50	4.70 ± 1.31	14.61 ± 1.52[d]	2.88 ± 2.09[a]
DCFD oxidation by blood PMNs (MESF, $\times 10^5$)	3.39 ± 0.43	5.90 ± 2.43[d]	0.86 ± 0.42	2.91 ± 2.48
SH rats				
PMA-stimulated chemiluminescence[c]	24.2 ± 13.7	24.5 ± 5.8	35.3 ± 10.0	22.0 ± 8.5
DCFD oxidation by BAL AMs (MESF, $\times 10^5$)	11.52 ± 5.00	9.00 ± 3.31	10.75 ± 2.76	9.89 ± 5.40
DCFD oxidation by blood PMNs (MESF, $\times 10^5$)	0.18 ± 0.06	0.13 ± 0.01	0.37 ± 0.04[d]	0.43 ± 0.04[a,d]

Note. Data are presented as means ± SD.
[a] Significantly different from LPS/shams, $p < .05$.
[b] Significant main effect of LPS, $p < .05$.
[c] PMA-stimulated chemiluminescence was measured in a suspension of mixed lavage cells.
[d] Significantly different from Saline/shams, $p < .05$.

(0.1–2.5 μm) without changes in pulmonary function (although the percentage of BAL PMNs increased). Unlike Seaton's work, we did not find any evidence in our studies for changes in packed red blood cell volume in either rat strain in response to inhaled laboratory-generated UFP. This could mean that components in ambient particulate pollution other than the carbonaceous core of UFP are responsible for such changes. In the compromised animals, however, our results do agree with those of Seaton in

TABLE 4
Exposure of old F-344 rats to systemic LPS ± inhaled ultrafine carbon particles: Summary of results

Parameters	UFP	LPS	UFP + LPS
BAL			
Total cell number	—	—	—
% PMNs	—	—	—
BAL cell chemiluminescence (stimulated)	—	$p = .03\downarrow$	—
BAL cell DCFD oxidation (AMs) (stimulated)	$p < .001\downarrow$	$p < .001\uparrow$	$p < .001\downarrow$
Blood			
Number of PMNs	$p = .01\downarrow$	$p < .001\uparrow$	$p = .02$
Blood cell DCFD oxidation (PMNs) (stimulated)	$p = .02\uparrow$	$p < .01\downarrow$	—
Hematocrit	—	—	—
Whole-blood viscosity	—	$p < .01\downarrow$	—
Plasma fibrinogen	—	$p < .001\uparrow$	$p < .01\uparrow$
Plasma TAT complexes	—	$p < .001\uparrow$	$p < .001\uparrow$
Plasma IL-6 concentration	—	$p = .02\uparrow$	—

Note. This table summarizes the statistically significant main effects and interactions found upon two-way ANOVA; p values are shown only for those effects that were significant; —indicates that no such effect was found. The arrows indicate the direction of the effect, relative to controls.

TABLE 5
Exposure of old spontaneously hypertensive rats to systemic LPS ± inhaled ultrafine carbon particles: Summary of results

Parameters	UFP	LPS	UFP + LPS
BAL			
Total cell number	—	—	—
% PMNs	—	—	—
BAL cell chemiluminescence (stimulated)	—	—	—
BAL cell DCFD oxidation (AMs) (stimulated)	—	—	—
Blood			
Number of PMNs	—	$p < .001\uparrow$	—
Blood cell DCFD oxidation (PMNs) (stimulated)	—	$p < .001\uparrow$	$p = .02\uparrow$
Hematocrit	—	—	—
Whole-blood viscosity	—	$p < .01\uparrow$	—
Plasma fibrinogen	$p < .001\downarrow$	$p < .001\uparrow$	—
Plasma TAT complexes	$p = .01\uparrow$	$p < .01\downarrow$	—
Plasma IL-6 concentration	ND	ND	ND

Note. This table summarizes the statistically significant main effects and interactions found upon two-way ANOVA; p values are shown only for those effects that were significant; a—indicates that no such effect was found. The arrows indicate the direction of the effect, relative to controls. ND, statistical analyses not done because samples from two of the four groups had undetectable levels of IL-6.

terms of particle-associated changes in fibrinogen: The combination of ip LPS with inhaled UFP resulted in lowered plasma fibrinogen.

Studies conducted in both humans and animal models show particle-associated increases in circulating PMNs (Clarke et al., 2000; Frampton et al., 2003). The particle types used in these studies span a wide range of size fractions and compositional signatures (concentrated ambient fine particles and laboratory-generated ultrafine particles). Interestingly, a study by Mukae and colleagues (2001) showed that repeated PM_{10} exposure accelerates the transit of immature PMNs through the bone marrow and into the circulation, thus increasing the number and percentage of band cells in blood, but without changing the overall number of blood PMNs. Although we found that the oxidative stress induced by ip LPS increased the number and percentage of peripheral PMNs in our studies, the effect of inhaled UFP was to cause a decrease in both rat strains. The disparity in these results may be related to several factors, including the length of exposure and the timing between the end of exposure and sampling of blood. The timing between exposure and sampling may be critical, as blood was drawn within 2 h at the most in both the Clarke and Frampton studies (∼18 h in ours). Our findings of UFP-associated decreases in blood PMNs may be related to altered adhesion between endothelial cells and circulating inflammatory cells. Indeed, our other studies in young and old mice have shown significant UFP-related decreases in both the surface expression of ICAM-1 on blood PMNs and on the number of PMNs themselves, suggesting that the ones expressing high levels of ICAM-1 are adhered to endothelial cells (Elder et al., 2002).

While we recognize that the genetic background of the SHR strain (Wistar Kyoto, WKY) is not the same as F-344 and that this difference could be a source of altered sensitivity to particles and LPS, all of our previous data were obtained from F-344 rats. Furthermore, this strain is extensively used in toxicological research and it was not within the scope of these studies to reevaluate ultrafine particle and ip LPS dose-response relationships in "normal" WKY rats. It is also the case that SHR and WKY rats have differences in their genotypes, despite the derivation of the hypertensive strain (Kodavanti et al., 1998). These genotypic differences could be the source of just as much variability in response as the strain differences. Having said this, though, there were some striking differences in response between the two rat strains that are noteworthy.

In vivo intracellular DCFD oxidation is not a common endpoint in animal studies. However, our results are interesting due to the main effects of particles that were found and the interactions between ip LPS and UFP in lavage and blood cells. In F-344 rats, we found that the effect of UFP was suppressive in terms of BAL cell DCFD oxidation; the same trend was present in SHRs, but the comparisons did not reach statistical significance. When LPS and UFP were combined in F-344 rats, the response was further suppressed. Aside from these differences, though, there was a heightened BAL AM DCFD oxidation in SHRs relative to F-344 rats that suggests that the lung cells from SH rats are at a higher level of oxidative stress; this notion is supported by work comparing the WKY and SHR strains from Schnackenberg and Wilcox (1999) and Suzuki et al. (1995). The lack of response in SHR to inhaled UFP may reflect an inability to respond to secondary stimuli. Blood PMN DCFD

oxidation was increased by inhaled UFP exposure, again either due to a main effect (F-344) or through an interaction with LPS (SHR). Similar to the strain differences already noted for BAL AMs, the overall 12-fold lower PMN DCFD oxidation in SHRs may represent a compensatory response to the enhanced levels of oxidative stress in this model or may reflect alternate pathways of response to stimuli as compared to normotensive rats (as suggested herein by responses to LPS that went in opposite directions in the two strains). Nevertheless, these data suggest that inhaled UFP can affect oxidative stress responses in blood and BAL cells and can interact with systemically-administered LPS.

As for plasma TAT complexes, we found that inhaled UFP increased their plasma levels in both rat strains, either through a main effect (SHR) or upon interaction with ip LPS (F-344). The highest response was to UFP alone in SHR; nevertheless, the combination of factors in SH rats still produced a higher response than in controls. Additionally, for all of the exposure groups taken as a whole, the plasma TAT complex concentration in SHRs is ~6.5 times higher than in F-344 rats (5-fold difference for controls alone). Increased plasma TAT complex concentration is indicative of thrombin generation (Chaiworapongsa et al., 2002; Ellison et al., 2001); the latter catalyzes the formation of fibrin monomers from fibrinogen. Thus, it would follow that elevations in TAT complexes, fibrinogen, or both would result in increased fibrin monomer production and clot formation. Indeed, elevations in plasma TAT complexes in humans are associated with arterial occlusions and intracoronary thrombi (Hoffmeister et al., 1995; Peltonen et al., 1995) as well as myocardial infarction and death in patients with unstable coronary artery disease (Oldgren et al., 2001). Despite higher average levels of TAT complexes and fibrinogen in SHRs, average values for whole blood viscosity were not different between the two strains. Viscosity may be under tight regulation in rats and therefore may not be as sensitive an indicator of coagulation as it is in humans. Interestingly, Peters et al. (1997) demonstrated an increase in plasma viscosity that was associated with increased mass concentrations of total suspended particulates in urban air. Presumably, ambient UFP would be present in high number concentrations and could be causally related to the observed changes in viscosity in humans.

Several studies have been conducted in the past few years by scientists at the U.S. EPA on the effects of emission particles (residual oil fly ash, ROFA) in SHRs. In one such study, Kodavanti et al. (2002a) found that plasma fibrinogen was elevated in SHRs, but not WKY rats, after 1 wk of inhalation exposures (15 mg/m^3); however, this effect did not persist with continued exposure. Increases in blood PMNs were also noted. Our results in SHRs exposed to laboratory-generated UFP do not agree with these findings, although there are some important differences between the two studies, which include the particle types, exposure concentration/duration, and rat age. ROFA consists of mainly fine-mode, metal-rich particles, which could induce effects through response pathways that are completely different from those activated by carbonaceous UFP. Second, the dose used in our studies was much lower than in the Kodavanti study (estimated total deposited dose in our studies was ~2% of their highest or 0.1% of their lowest dose). These exposures were also performed on 3 days/wk for up to 4 wk, whereas the results presented herein are from a single 6-h exposure. Lastly, we used aged (11–14 mo) SHR as opposed to young adults (12–13 wk). Nevertheless, one particularly interesting finding of this group was that ROFA particles were cleared from the lungs to a greater extent in SHRs versus WKY rats and were found in the interstitium and lung-associated lymph nodes, a typical finding in lung particle overload; therefore, this may be related to the high dose used (Kodavanti et al., 2002b). This "leakiness" of the lungs from SHRs was also shown in our studies (higher protein concentrations, more RBCs or hemolyzed blood products in BAL fluid) and appears to be a common feature of the SH rat model that is unrelated to particle exposure or their interstitial translocation per se.

In summary, these results suggest that inhaled ultrafine carbon particles at concentrations mimicking high episodic events can independently induce systemic responses and that interactions between these particles and systemically administered LPS occur in both the pulmonary and extrapulmonary compartments. One intriguing result is that plasma TAT complexes are increased by UFP in both animal models, either through a main effect of the particles themselves or via an interaction with LPS. The study also highlights the heightened oxidative stress in SHRs, as shown by differences in basal levels of BAL AM and blood PMN DCFD oxidation between the strains. The extent to which the observed effects and interactions are particle size specific and composition specific should be examined in the future to aid in the prediction of outcome in humans exposed to ambient air particulate matter.

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